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*Published in:*
Physiological and Biochemical Zoology

*DOI:*
10.1086/319314

# The Effect of Food Restriction on Morphological and Metabolic Development in Two Lines of Growing Japanese Quail Chicks

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Accepted 8/25/00

## ABSTRACT

To investigate whether there is a relationship between the inherent maximum growth rate of a chick and its degree of developmental plasticity, we studied the effects of food restriction on morphological and physiological development in P-line (selected for high growth rates) and C-line (nonselected) chicks of the Japanese quail between 2 and 64 d of age. In each line, three groups were created; one group in which the chicks were fed ad lib. and two other groups in which the chicks received 70% and 40% of the line-specific ad lib. feeding levels, respectively. In all three groups, chick survival rates were very high, and no relationship was detected between feeding level and chick mortality. The experimental feeding levels strongly affected the chicks' attainment of body mass at all ages. In addition, at most ages the growth of the structural components (wing, tarsus, and head) were also significantly affected by the feeding experiment but to a much smaller extent than the body mass. The feeding levels also affected the achievement of female sexual maturity such that, at the end of the experiment, most of the ad lib.–fed females and none of the females that received 40% of the ad lib. feeding level were laying eggs. In chicks of both lines that were fed ad lib., homeothermy was achieved at 7 d of age, but in chicks that received 40% of the line-specific ad lib. levels, homeothermy was achieved after 15–22 d of age. Poikilothermic chicks (P- and C-line) that were relatively heavy for their age exhibited relatively high resting metabolic rates (RMRs) and peak metabolic rates (PMRs) for their age. In addition, a positive relationship was found between the chicks' relative mass and RMR (based on mass-based predictive equations). No relationship was detected between the chicks' relative body mass and their relative PMR (relative to mass-based predictive equations). With respect to the metabolic scope (i.e., PMR − RMR), Japanese quail chicks exhibited remarkable developmental plasticity: chicks with body masses that were 50% lower than the normal growing chicks did exhibit a normal capacity of thermogenic heat production for their mass. Because no differences were found with respect to the level of plasticity of morphological and physiological development between the two strains, we conclude that the selection for high postnatal growth rates in P-strain chicks has not resulted in a in a higher vulnerability of these chicks to food restrictions.

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*Physiological and Biochemical Zoology* 74(1):52–65. 2001.  1522-2152/2001/7401-99106$03.00

## Introduction

Growing chicks are frequently exposed to variations in their food supply (Schew and Ricklefs 1998). To maximize their survival under poor feeding conditions, chicks should optimize the allocation of their ingested energy to growth and maintenance. To reduce the impact of food restrictions, chicks have developed a suite of behavioral, morphological, and physiological adaptations. Although much information has been collected with respect to the plasticity of behavioral, morphological, and physiological development, virtually nothing is known about the underlying mechanisms that affect the degree of the chicks' developmental plasticity (Schew and Ricklefs 1998).

In the field, time budgets of precocial chicks consist of alternating bouts of foraging freely and being brooded by a parent. During bad weather conditions especially, the amount of time available for foraging can be strongly reduced, which potentially leads to a reduction of food intake and to a suppression of normal growth (Beintema and Visser 1989*a*, 1989*b*; Visser 1998). Such a reduction in growth rate may also affect the chick's developmental pattern of metabolism and temperature regulation as well as its rate of sexual maturation (Morse and Vohra 1971; Klaassen and Bech 1992; Harun et al. 1997). To maximize its growth rate, and probably its survival, a chick should minimize its level of daily energy expenditure, enabling it to maximize the allocation of energy to growth (Klaassen 1994; Kersten and Brenninkmeijer 1995; Weathers 1996). At reduced levels of energy intake, the remaining energy for growth can either be allocated to all different body components at similar proportions as in chicks that are fed ad lib. (but at lower rates) or be specifically allocated to some favored body components at the expense of other components (Schew and Ricklefs 1998).

There is considerable evidence that the degree of plasticity of morphological and physiological development differs among species (Konarzewski et al. 1996; Schew and Ricklefs 1998). For example, in young European starling chicks (*Sturnus vulgaris*, altricial mode of development), when fed at the maintenance level, the growth rate of tarsus and wing chord only gradually decreased resulting in a depletion of their energy reserves, whereas in young Japanese quail chicks (*Coturnix coturnix japonica*, precocial mode of development), growth of these structural components almost immediately stopped (thereby maintaining their body reserves; Schew and Ricklefs 1998). Precocial species especially exhibit a remarkable degree of flexibility with respect to the rate of growth and maturation in response to reduced food intake (for a recent review, see Schew and Ricklefs 1998). Moreover, it is well known that, at a given asymptotic body mass, precocial chicks exhibit much lower growth rates than altricial chicks (Ricklefs 1979; Starck and Ricklefs 1998). Therefore, it is tempting to speculate whether the developmental plasticity is in some way related to the inherent maximum growth rate of the chick.

To investigate the idea that the level of the chicks' developmental plasticity is related to their inherent maximum growth rate, we compared two different lines of the Japanese quail: one line that has been selected for a high postnatal growth rate (P-line) and a nonselected line (C-line). In each line we created one group in which the chicks were fed ad lib. (henceforth abbreviated as P100 and C100, respectively). Chicks of the other groups received 70% (P70 and C70, respectively), and 40% (P40 and C40, respectively) of the predetermined age- and line-specific ad lib. levels of gross energy intake. We determined development of body mass and the growth of several structural components (i.e., tarsus, wing, and head), as well as the development of temperature regulation and metabolism such as resting metabolic rate at thermoneutrality and peak metabolic rate. In addition, in females we monitored the onset of egg production. By applying this food restriction protocol, we were able to disentangle the separate contributions of mass and age on the development of metabolic rate, homeothermy, and the onset of sexual maturation in chicks with contrasting inherent growth rates (Klaassen and Bech 1992). If the developmental plasticity is in some way related to the chicks' inherent maximum growth rate, then we would expect significant differences in the responses of the chicks to food restrictions.

## Material and Methods

### *Animals, Housing, and Experimental Design*

The food restriction experiment was performed in chicks of two Japanese quail lines. Chicks of one line have been selected for rapid growth for about 40 generations (P-line), and chicks of the other line were nonselected (C-line; Nouwen B.V., Lommel, Belgium). For the experiments, only chicks that were hatched on the same day (designated as day 0) were used. At the end of this day (at about 1700 hours), the dry chicks were weighed on a Mettler PT 2010N balance to the nearest 0.1 g, banded with a numbered aluminium ring, and housed in groups of eight in wooden cages (l × w × h: 0.67 × 0.39 × 0.44 m) with sawdust bedding. Food and water were available ad lib. with lights on during 24 h. In each cage, an infrared heating lamp (60 W) was placed at one end of the cage to provide a temperature gradient throughout the cage; this provided sufficient heat for the chicks to maintain a normal body temperature.

At day 2, the chicks were weighed again at about 0900 hours. Thereafter, the experimental feeding levels were set at 100%, 70%, and 40% of the predetermined age-specific and line-specific ad lib. feeding levels (Boon et al. 2000; see Fig. 1, *upper panels*), resulting in three groups in the P-line (P100, P70, and P40, respectively), and three groups in the C-line (C100, C70, and C40, respectively). Water was always supplied ad lib. The chicks were housed in the wooden cages with two or three individuals per cage with the infrared heating lamp but with a mesh wire bottom. We decided to apply these housing conditions because pilot experiments revealed that individually housed chicks were very restless, which potentially interferes with normal growth. From then onward the birds were subjected to an 18L : 6D cycle (lights on at 0900 hours). The chicks were given food pellets once a day (between 0900 and 1000 hours) containing 27.7% (w/w) crude protein and a gross energy content of 17.0 kJ $g^{-1}$ wet matter (P. Boon, personal communication). The feeding trays were constructed such that the spilled food was still available for the birds. The experiment lasted until 64 d of age, when the birds achieved asymptotic levels with respect to the length of wing, tarsus, and head. The whole experiment was performed twice. In total, 68 chicks were used with 10–12 chicks in each experimental group.

### *Morphological Measurements and Determination of Egg Production*

All measurements of body mass and the structural components were always taken between 0900 and 1000 hours. Between the hatching day and day 30, body mass was measured daily and, thereafter, twice a week (to the nearest 0.1 g, Mettler PT 2010N). During the first week, the length of the tarsus, wingchord, and head plus bill (henceforth abbreviated as head) were determined to the nearest 0.5 mm every other day using a dial caliper (Kersten and Brennikmeijer 1995). During the following 3 wk, these measurements were taken three times a week and then once a week during the remainder of the experimental period (always by the same person). For older chicks, the cages were inspected daily on the presence of eggs.

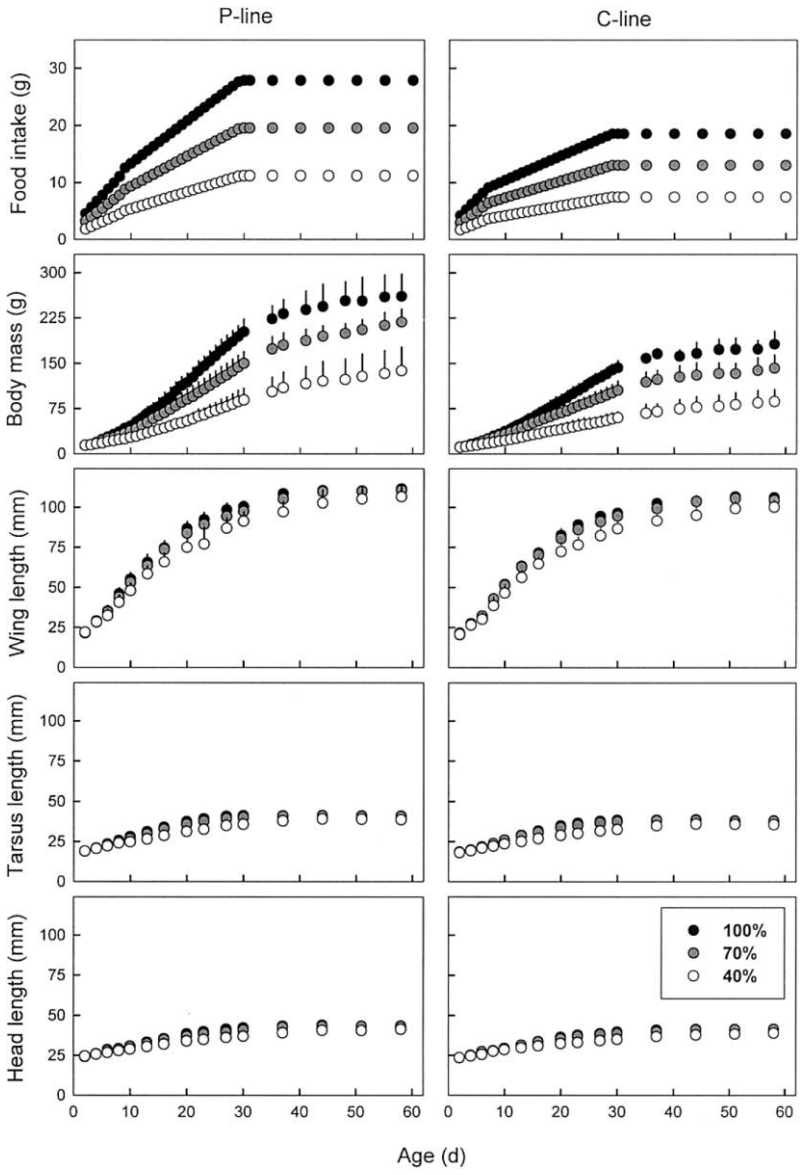


Figure 1. The development of food intake level (g), body mass (g), wing length (mm), tarsus length (mm), and head length (mm) with age in the P- (*left*) and C-line (*right*) quail chicks (mean values presented and SE). For most of the points, the standard error bars fall within the symbol of the mean. Filled circles represent chicks fed ad lib. Gray and open circles represent chicks that received 70% or 40% of the ad lib. levels, respectively.

*Metabolic Measurements*

During the daytime (between 1230 and 1900 hours), we determined resting metabolic rate at thermoneutrality and peak metabolic rate in response to cold exposure (for definitions, see Visser 1998). The metabolic rate measurements were performed by means of indirect calorimetry (for details about the experimental design, see Krijgsveld et al. 1998). We could simultaneously measure metabolic rates in five separate chicks. To avoid a potential bias in the nutritional status among chicks of the different groups, at first each chick had access to food during 10 min, and it was subsequently fasted for about 3 h before the measurements. Next, the chick's body temperature was determined to the nearest 0.1°C by inserting a Therm 2244-1 temperature probe about 2 cm into the rectum, and its body mass was determined on a Sartorius PT120 balance. Thereafter,

the chick was placed individually in a metabolic chamber (l × w × h: 0.15 × 0.10 × 0.11 m for chicks up to about 100 g and 0.35 × 0.25 × 0.25 m for heavier chicks). Last, the metabolic chamber was placed in a darkened temperature cabinet (Heraeus) with the ambient temperature set at 35°C (for chicks up to about 100 g) or 30°C (for heavier chicks). These temperatures all fall within the thermoneutral zone of the birds (G. H. Visser, P. Boon, and C. E. van der Ziel, unpublished data). Dried compressed air was passed under pressure through each respiration chamber. The flow rate was measured on the inlet air (Brooks 5850E, with maximum capacities of 60 and 300 L $h^{-1}$). Flow rates changed with age and were adjusted such that the expected minimum $O_2$ and maximum $CO_2$ concentrations of the outflowing air were about 20.2% and 0.6%, respectively. Mass flow controllers were calibrated regularly with a soap foam flowmeter (Bubble-O-Meter, La Verne, Calif.). The outflowing air was dried over a tube filled with molecular sieve (3 Å, Merck). Once every 6 min, the outlet air of a given respiration chamber was flushed for 1 min through the $CO_2$ and $O_2$ gas analyzers (Binos infrared $CO_2$ analyzer, Leybold Heraeus, and a S-3A/II Oxygen analyzer, Applied Electrochemistry, respectively). The reading for that specific chamber was made during the last 10 s of the 1-min flushing period. Thereafter, the respiration gas of another respiration chamber was passed through the gas analyzers. In addition, every 6 min a sample of the inlet air was passed through the analyzers to determine its $O_2$ and $CO_2$ concentrations.

After two measurement hours at thermoneutrality, the temperature in the cabinet was lowered at a constant rate of 0.2°C $min^{-1}$. After some time, this resulted in a gradual increase of the $O_2$ consumption and $CO_2$ production (caused by thermogenic heat production of the chick) to achieve plateau values for each gas (indicating the metabolic rates could not be elevated in response to a decrease of the ambient temperature). After a further decrease in ambient temperature, $O_2$ consumption and $CO_2$ production often strongly decreased, indicating that metabolic rates had gone down because of body cooling, and the measurement process was stopped. At the end of the measurement process, body mass and body temperature were determined again as indicated above. Body mass during the measurement was considered to be the average value of body mass at the start and end. The minimum ambient temperature that could be achieved with the apparatus was about −5°C. Because older chicks exhibit peak metabolic rate (PMR) only at very low ambient temperatures (Visser 1998), PMR determinations with our apparatus appeared to be only possible in chicks up to about 30 g in chicks of the 100% and 70% groups and up to about 50 g in chicks of both 40% groups. In heavier chicks, resting metabolic rate (RMR) measurements were performed during 2-h trials at thermoneutrality (for temperature conditions and feeding conditions before the measurement, see above).

The data files for each individual included the chamber temperature, flow rate, and $O_2$ and $CO_2$ concentrations of the inlet and outlet air at 6-min intervals. Rates of $O_2$ consumption and $CO_2$ production (both in L $h^{-1}$ STPD) were calculated according to Hill (1972). In addition, calculated gas exchange rates were corrected for changes in the gas concentrations during the measurements by taking into account the size of the metabolic chambers (Bartholomew et al. 1981). The respiration quotient (RQ) was calculated as the ratio of $CO_2$ production and $O_2$ consumption and was always below 0.75, indicating that the bird was fasting during the measurement.

We defined the level of the resting metabolic rate at thermoneutrality (RMR, amount of $O_2$ consumed in L $h^{-1}$) as the lowest value of the 18-min running average of the measurements. Peak metabolic rate (PMR, amount of $O_2$ consumed in L $h^{-1}$) was defined as the maximum 12-min running average (Visser and Ricklefs 1993). Birds were considered to have been at their peak level if body temperatures had dropped below 35°C at the end of the measurement (following Klaassen and Bech 1992). The chicks were considered to be homeothermic if their body temperatures were at least 35°C at the end of the measurement. In poikilothermic chicks, metabolic scope, in liters $O_2$ consumed $h^{-1}$ (henceforth abbreviated as SCOPE), was calculated as PMR − RMR. Between 2 and 10 d of age, metabolic measurements were performed daily for at least one chick in each group. Thereafter until about day 30, the measurements were performed weekly. The last measurements were made between 50 and 64 d of age. The PMR of each chick was never measured at more than two different ages, and its RMR at never more than three different ages.

For each animal, for each measurement, we calculated the residual body mass at a certain age ($M_{res}$, percentage) by comparing its actual measured body mass during the measurement ($M$, g) with the average age- and line-specific ad lib. body mass ($M_{avg}$, g):

$$M_{res} = 100 \times \frac{(M - M_{avg})}{M_{avg}}. \quad (1)$$

*Statistics*

The measurements were analyzed by means of SPSS/PC 5.01 (Norušis 1992) using a probability level of $P < 0.05$ to determine statistical significance. ANOVA was used to compare structural body components of the three different feeding groups. Logistic regression was used to test the effect of treatment on egg-laying performance at 60 d of age. ANCOVA was applied to compare the allometric relationships between RMR or PMR and body mass for both lines and the three feeding levels.

## Results

### *Chick Survival*

In all groups, survival rates were very high, only in the P40 group did one chick accidentally die. In addition, in the C100 group, three chicks had to be removed from the experiment because of an early onset of excessive aggression. The data of these four birds were deleted from the data set. On the basis of these high survival rates, it can be concluded that the chicks managed to cope with the food restrictions.

### *Development of Body Mass*

The experimental feeding levels strongly affected the chicks' development of body mass (Fig. 1). On day 60, the body masses of birds of the P-line differed significantly within each line and were on the average 260.3 g (SD = 35.5), 217.6 g (SD = 20.4; i.e., at 83.6% of the P100 level), and 137.7 g (SD = 36.7; i.e., at 52.9% of the P100 level) for the P100, P70, and P40 groups, respectively (ANOVA, $P < 0.01$). For the C100, C70, and C40 groups, the corresponding values were 181.0 g (SD = 20.4), 141.7 g (SD = 20.9; i.e., 78.3% of the C100 level), and 86.4 g (SD = 19.0; i.e., 47.7% of the C100 level), respectively (ANOVA, $P < 0.01$). Within each strain, the body mass differences between the three groups are not entirely proportional to the relative feeding levels. This can be caused by differences in egg production between the three groups (see "Discussion"), differences in activity, or differences in digestion efficiencies between the three groups (Lepczyk et al. 1998).

In chicks of the three groups of the P-line, daily growth rates peaked between 15 and 30 d of age. Values were highest in P100 (about 10 g $d^{-1}$), intermediate in P70 (about 6 g $d^{-1}$), and lowest in P40 chicks (about 4 g $d^{-1}$; Fig. 2). At older ages, daily mass increments were often higher in P40 and P70 chicks compared with those of the P100 chicks, which resulted in a slight decrease in the differences in body masses between the three groups during the latter part of the experiments (see Fig. 1). A similar pattern could be observed in chicks of the C-line, with maximum growth rates for C100, C70, and C40 chicks of about 6, 4, and 2 g $d^{-1}$, respectively (Fig. 2).

### *Development of the Length of the Wingchord, Tarsus, and Head*

In chicks that received 40% of the ad lib. levels, growth of all structural components continued after the start of the food restriction experiments, but at lower rates initially than chicks that were fed ad lib. (Fig. 2). However, the differences between the three feeding groups for the structural components were not as large as the observed differences between body mass (Figs. 1, 2). For example, at day 30, wing lengths of the P70 and P40 chicks were on average 96.9% and 90.7% of the ad lib. levels, respectively, and wing lengths of the C70 and C40 chicks were on average 98.1% and 89.8% of the ad lib. levels, respectively. At older ages, these differences gradually decreased, and at 60 d of age, values for the P70 and P40 chicks were not significantly different from the P100 chicks. The values for the C70 and C40 chicks were at about 98.3% and 94.47% of the ad lib. levels, respectively (Fig. 1).

Similar to the analysis of development of body mass, we calculated the daily growth rates for each component (Fig. 2). In contrast to development of body mass, growth rates of the three structural components already peaked between 4 and 10 d of age. Until about 20 d of age, growth rates of P40 and C40 chicks fell well below those of the other groups. However, beyond this age, growth rates of the P40 and C40 chicks exceeded those of the other groups, which explains why the differences in structural components between the different groups decreased toward the end of the experiments (Fig. 1).

### *Egg Production*

First eggs were produced at 43 d of age (in P100, C100, and C70 females). At 50 d of age, for the combined data set of the P and C females, it was found that in the ad lib.–fed birds, five of 11 females were laying eggs, in the 70% birds one out of 14, and in the 40% birds none of 10 females. At 60 d of age, the values for the three different groups were six (out of 11), three (out of 14), and 0 (out of 10). At this age, logistic regression analysis for the pooled data on birds of the P and C line revealed that that there was a significant relationship between the feeding level (abbreviated as RFL; i.e., 40%, 70%, and 100% of ad lib. feeding) and the achievement of female sexual maturity:

$$\log\left(\frac{\text{egg-laying females}}{\text{non-egg-laying females}}\right) = -5.96 + 6.25 \times \text{RFL} \quad (2)$$

(SE constant = 2.126, SE slope = 2.461, $P < 0.017$). Unfortunately, because of the fact that in some cases two females were housed in the same cage, we were unable to identify which female was actually laying. Therefore, we were unable to assess the female's body mass at the onset of egg laying.

### *Development of RMR and PMR*

In all experimental groups, in chicks until about 30 d of age there was a more or less linear increase in RMR with age, and thereafter values gradually leveled off (Fig. 3). However, when RMR was graphed against body mass (on a doubly logarithmic basis), metabolic rates increased over the entire range (Fig. 4). PMR levels increased even more strongly with age (Fig. 3) and body mass (Fig. 4). In P100 and C100, chicks we were unable to measure PMR in chicks older than 7 d because of the achievement of homeothermy at ambient temperatures of –5°C (see

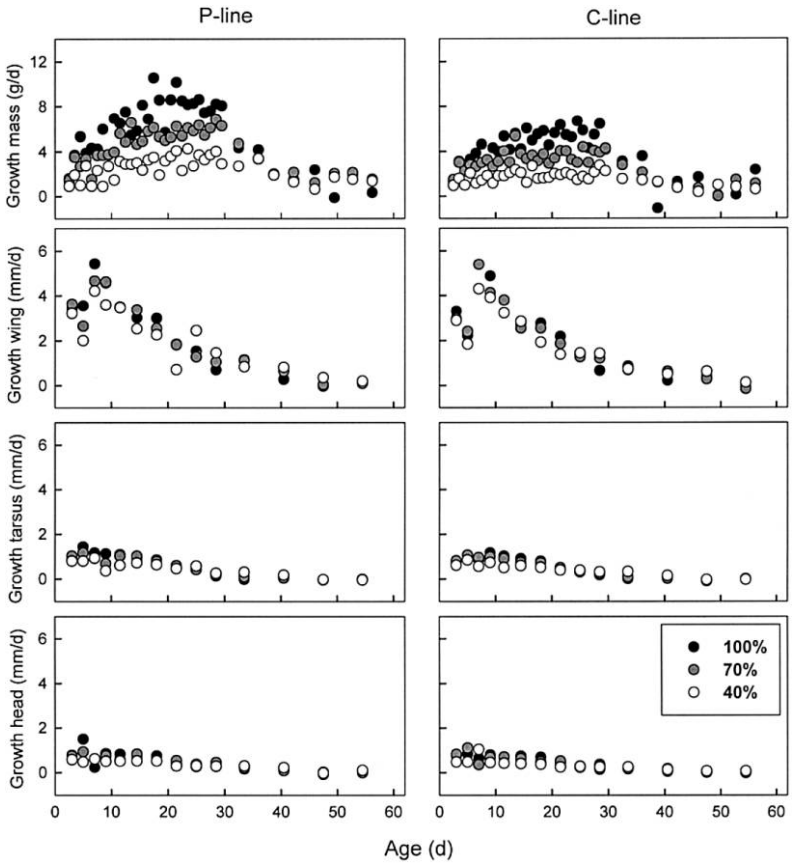


Figure 2. Daily growth rates of the body mass, wing, tarsus, and head in relation to age in chicks of the Japanese quail of the P-line (*left*) and of the C-line (*right*). For explanation of the symbols, see the legend of Figure 1.

"Material and Methods"). However, in P40 and C40, chicks' homeothermy was not achieved until 15 or 22 d of age, respectively. In the subsequent sections on development of metabolism and temperature regulation, the data set has been subdivided into the poikilothermic phase (measurements of RMR, PMR, and SCOPE) and the homeothermic phase (measurements of RMR only).

*Poikilothermic Chicks: Age-Based Regressions of RMR, PMR, and SCOPE*

For each line, different analyses were performed to disentangle the individual contributions of age and body mass to the development RMR, PMR, and SCOPE (Klaassen and Bech 1992). For each line, for the combined data set of the 100%, 70%, and 40% groups, relationships between RMR and age could be described by linear regressions (Table 1; data set displayed in Fig. 3). In chicks of the P-line, the separate relationships between PMR and age and SCOPE and age could be described with a linear model. However, in chicks of the C-line, for both relationships the addition of a quadratic term significantly improved the explained variance (Table 1). However, relationships between age and RMR, PMR, or SCOPE exhibited low coefficients of determination (range $r^2$ values: P-line, 0.38–0.57; C-line, 0.49–0.67), suggesting that factors other than age may also have affected the levels of these parameters. Therefore, for each RMR measurement, its residual value ($RMR_{res}$, percentage) was calculated with the following equation:

$$RMR_{res} = 100 \times \frac{(\text{measured RMR} - \text{predicted RMR})}{\text{predicted RMR}}. \quad (3)$$

The predicted RMR values are based on the line-specific age-based equation listed in Table 1. In chicks of the P-line used for the metabolic measurements, body mass residuals ranged from −56.7% to 16.2%, and in chicks of the C-line they ranged from −58.3% to 8.6%. Analysis revealed that within each line, a significant relationship existed between the relative body mass of a chick ($M_{res}$, value obtained using Eq. [1]) and its relative

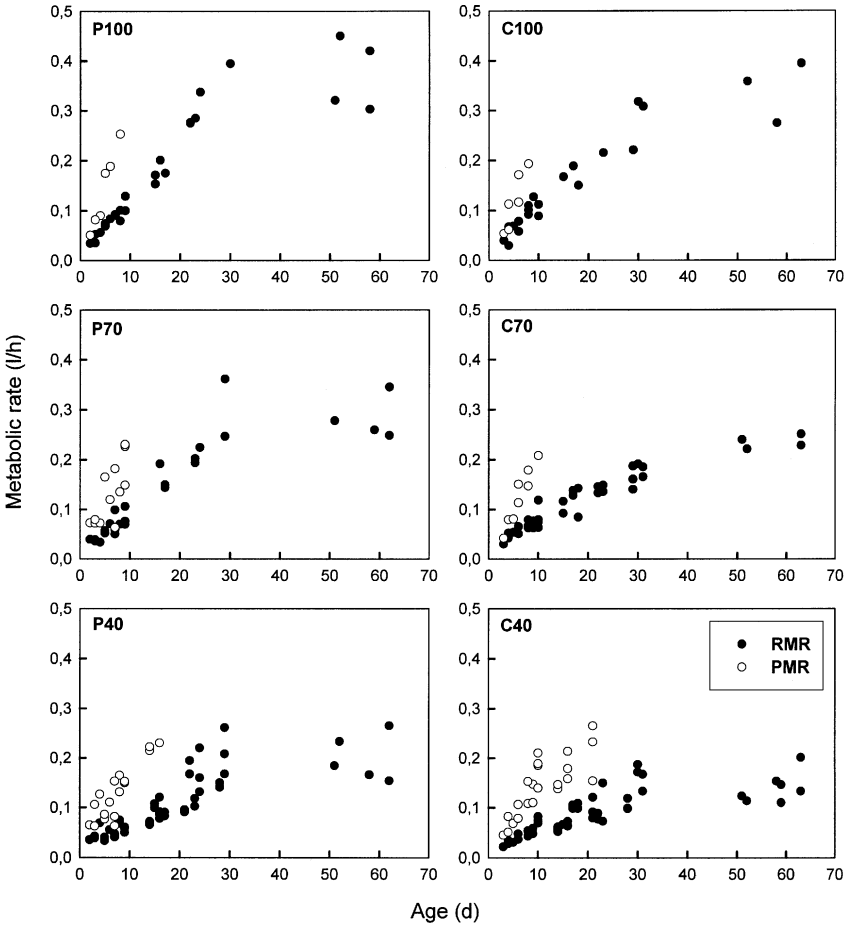


Figure 3. Relationships between resting metabolic (*filled circles*) and peak metabolic rates (*open circles*) with age in Japanese quail chicks of the P-line (*left*) and of the C-line (*right*).

RMR (i.e., $RMR_{res}$). In chicks of the P-line this relationship could be described by

$$RMR_{res} = 9.42 + 0.46 \times M_{res} \quad (4)$$

(SE intercept = 5.51, SE slope = 0.188, $r^2 = 0.15$, $P = 0.02$, $n = 36$; Fig. 5), and in chicks of the C-line by

$$RMR_{res} = 18.23 + 0.68 \times M_{res} \quad (5)$$

(SE intercept = 7.67, SE slope = 0.235, $r^2 = 0.20$, $P < 0.01$, $n = 36$; Fig. 5). Thus, for both lines it can be concluded that chicks that were relatively heavy for their age exhibited relatively high RMR values for their age.

In a similar way as that outlined in Equation (3), we calculated for each PMR measurement its residual value ($PMR_{res}$, percentage) relative to the aforementioned predictive equations for PMR listed in Table 1. In chicks of the P-line, the relationship between $PMR_{res}$ and $M_{res}$ could be described by

$$PMR_{res} = 10.94 + 0.55 \times M_{res} \quad (6)$$

(SE intercept = 6.71, SE slope = 0.229, $r^2 = 0.14$, $P < 0.023$, $n = 36$; Fig. 6), and in chicks of the C-line by

$$PMR_{res} = 15.78 + 0.61 \times M_{res} \quad (7)$$

(SE intercept = 6.85, SE slope = 0.211, $r^2 = 0.20$, $P < 0.01$, $n = 36$; Fig. 6). Thus, from these analyses it can be concluded that chicks that are heavy for their age exhibited high PMR values for their age. However, the actual PMR value of a chick includes both the RMR and SCOPE. Therefore, in a similar way as that outlined in Equation (3), we calculated for each SCOPE value its residual value ($SCOPE_{res}$, percentage) relative

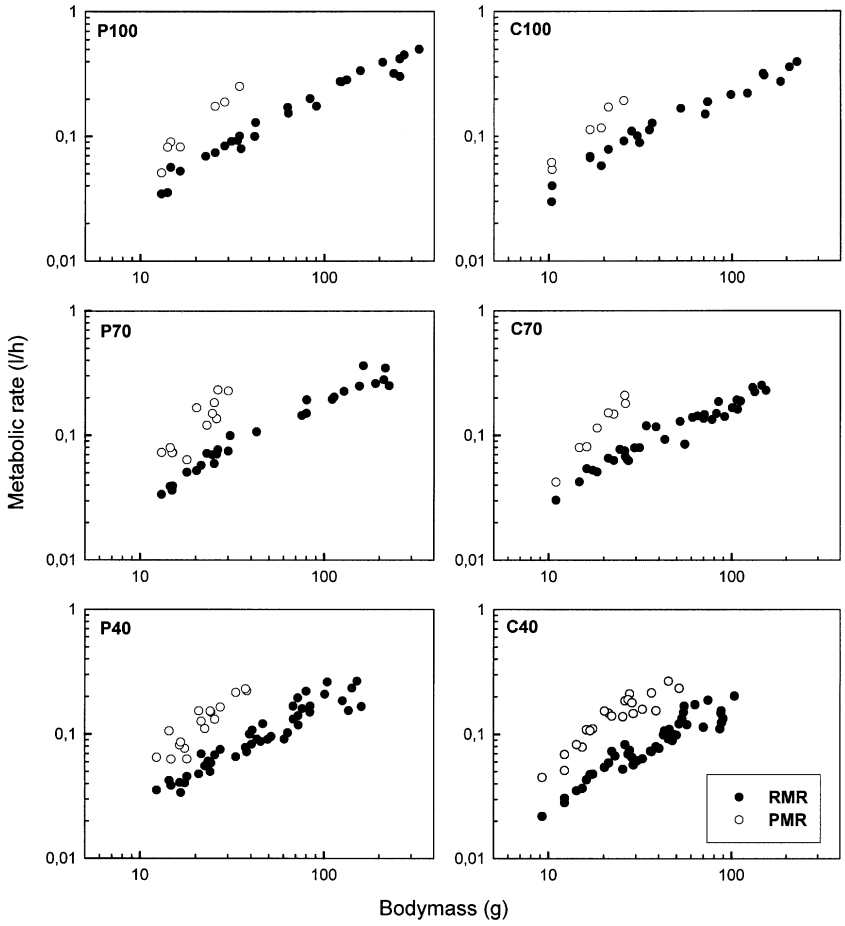


Figure 4. Allometric relationships between resting metabolic rate (*filled circles*) and peak metabolic rate (*open circles*) with body mass in Japanese quail chicks of the P-line (*left*) and of the C-line (*right*).

to the aforementioned predictive age-based equations for SCOPE listed in Table 1. The relationship between $SCOPE_{res}$ and $M_{res}$ appeared not to be statistically significant in chicks of the P-line ($P = 0.07$, $n = 36$; Fig. 7), nor of the C-line ($P = 0.07$, $n = 36$; Fig. 7). Thus, the high $PMR_{res}$ values in birds with a high $M_{res}$ (as demonstrated above) was caused by a high $RMR_{res}$ and not by a high $SCOPE_{res}$.

*Poikilothermic Chicks: Mass-Based Regressions of RMR, PMR, and SCOPE*

To investigate the effect of body mass on development of RMR, PMR, and SCOPE, the logarithm of the metabolic measurement was related to the logarithm of the chick's body mass (for the line-specific parameter estimates, see Table 1; Fig. 4). Similar to the procedure described above, for the combined data set of the 100%, 70%, and 40% groups, for each line separately, relationships between $\log_{10}$ (RMR) and $\log_{10}(M)$ could be described adequately by linear regressions (Table 1). However, in some cases the addition of a quadratic term significantly improved the fit (see also Weathers and Siegel 1995). For the separate relationships, coefficients of determination ranged between 0.65 and 0.91. In search for factors that could explain the remaining variance for each RMR measurement the value was calculated ($RMR_{res}$, percentage) using Equation (3), but now on the basis of the mass-based predictive equations (after back-transformation of the log-based predictions).

For chicks of the P-line, the relationship between $RMR_{res}$ and $M_{res}$ could be described as follows

$$RMR_{res} = 7.21 + 0.30 \times M_{res} \quad (8)$$

(SE intercept = 3.18, SE slope = 0.109, $r^2 = 0.18$, $P < 0.01$, $n = 36$; Fig. 5). For chicks of the C-line, this relationship could be described by

$$RMR_{res} = 14.71 + 0.48 \times M_{res} \quad (9)$$

Table 1: Line-specific parameter estimates (mean and SE) for the relationships between body mass or age with resting metabolic rate (RMR), peak metabolic rate (PMR), and metabolic scope (SCOPE) in poikilothermic chicks

| | *a* | *b* | *c* | $r^2$ | *P* |
|---|---|---|---|---|---|
| P-line ($n = 36$): | | | | | |
| Age-based regressions:[a] | | | | | |
| RMR | .0362 | .00313 (.000687) | | .38 | .0001 |
| PMR | .0464 | .01310 (.001974) | | .57 | .0001 |
| SCOPE | .0102 | .00997 (.001538) | | .55 | .0001 |
| Mass-based regressions:[b] | | | | | |
| RMR | −2.402 | .86094 (.074586) | | .80 | .0001 |
| PMR | −2.665 | 1.31875 (.102314) | | .83 | .0001 |
| SCOPE | −3.509 | 1.7385 (.2175) | | .65 | .0001 |
| C-line ($n = 36$): | | | | | |
| Age-based regressions:[a] | | | | | |
| RMR | .0334 | .00284 (.000496) | | .49 | .0001 |
| PMR | −.00164 | .02276 (.004909) | −.00062 (.000206) | .66 | .0001 |
| SCOPE | −.02652 | .01797 (.003361) | −.00054 (.000141) | .67 | .0001 |
| Mass-based regressions:[b] | | | | | |
| RMR | −3.492 | 2.607 (.8052) | −.679 (.4042) | .82 | .0001 |
| PMR | −4.502 | 4.440 (.7343) | −1.276 (.2774) | .91 | .0001 |
| SCOPE | −6.359 | 6.604 (1.3468) | − 1.994 (.5088) | .83 | .0001 |

[a] Equation used: Factor $= a + b \times \text{age} + c \times \text{age}^2$ (age in days). Quadratic term only included if this significantly improved the fit.

[b] Equation used: Factor $= a + b \times [\log_{10}(M)] + c \times [\log_{10} M]^2$ ($M$ in grams). The quadratic term was only included if this significantly improved the fit ($P < 0.05$).

(SE intercept = 4.68, SE slope = 0.143, $r^2 = 0.25$, $P < 0.01$, $n = 36$; Fig. 5). This suggests that chicks that have low body masses for their age (i.e., birds with low body mass residuals) exhibit low RMR levels for their body mass.

Mass-based predictive equations for PMR and SCOPE are listed in Table 1. Residual values ($PMR_{res}$ and $SCOPE_{res}$ [units: percentages] were calculated using Equation [3] after back-transformation of the log-based predictions). The relationship between $PMR_{res}$ and $M_{res}$ was not significant for chicks of either the P-line ($P = 0.33$; Fig. 6) or the C-line ($P = 0.28$; Fig. 6). This suggests that, for both lines, there was no relationship between the relative body mass of the chick and its PMR relative to the mass-predicted value. Thus, at a given body mass, chicks that exhibit low body masses for their age (i.e., with a low body mass residual) exhibit PMR levels similar those of to chicks that are relatively heavy for their age (i.e., with a high body mass residual). Similarly, we were not able to demonstrate a significant relationship between $SCOPE_{res}$ and $M_{res}$ in chicks of either the P-line ($P = 0.71$, $n = 36$; Fig. 7) or the C-line ($P = 0.59$, $n = 36$; Fig. 7). This result suggests that over a very wide range of relative body masses, development of PMR or SCOPE proceeds at a rate that is closely linked to the actual body mass but not to the relative body mass.

*Is There a Link between the RMR Level and Metabolic Scope?*

The SCOPE level has been considered to be a measure of the degree of functional maturity of the chick's muscles that contribute to thermogenesis (Ricklefs 1979). Moreover, chicks that exhibit high SCOPE levels may require a larger metabolic machinery (such as the stomach, gastrointestinal tract, liver, and intestines) to support such high levels of energy expenditure (Klaassen and Drent 1991). Because these tissues exhibit high mass-specific metabolic rates, a capacity to sustain high PMR levels may also lead to an elevation of the chick's RMR. Therefore, regression analysis was performed to investigate whether a relatively high $SCOPE_{res}$ level (mass based) in a chick is accompanied by a relatively high $RMR_{res}$ level (mass based). In chicks of the P-line, this relationship could be described by

$$SCOPE_{res} = 7.86 - 0.37 \times RMR_{res} \quad (10)$$

(SE intercept = 6.596, SE slope = 0.464, $F_{1,34} = 0.62$, $P = 0.44$, $r^2 = 0.018$) and in chicks of the C-line by

$$SCOPE_{res} = 3.87 - 0.19 \times RMR_{res} \quad (11)$$

(SE intercept = 4.466, SE slope = 0.263, $F_{1,34} = 0.51$, $P =$

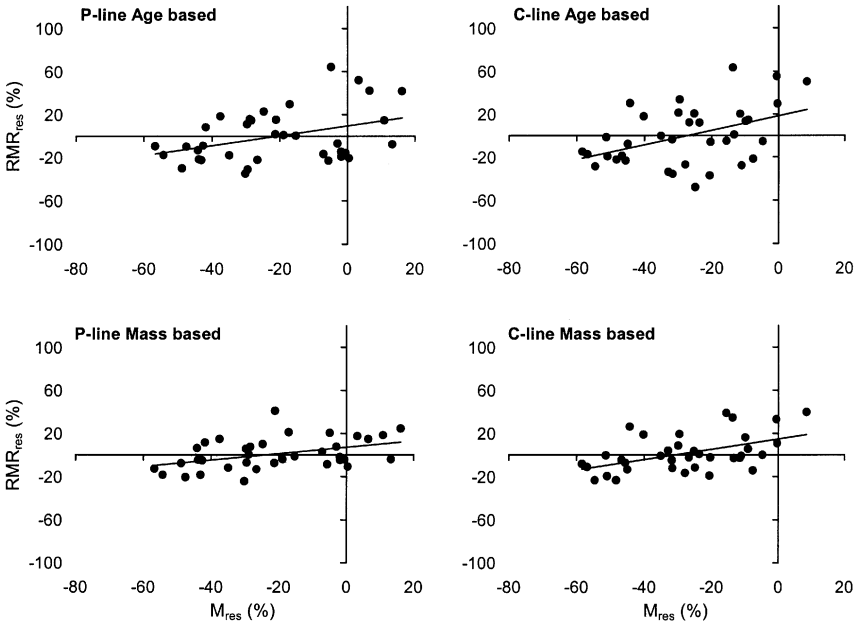


Figure 5. Age- (*upper panels*) and mass-based (*lower panels*) regressions of resting metabolic rate residuals ($RMR_{res}$) and body mass residuals ($M_{res}$) in Japanese quail chicks of the P- and C-line. For P-line chicks, age-based regression refers to Equation (4), and mass-based regression to Equation (8). For C-line chicks, age-based regression refers to Equation (5), and mass-based regression to Equation (9).

0.48, $r^2 = 0.015$). The absence of a relationship between $SCOPE_{res}$ and $RMR_{res}$ suggests that a high SCOPE level can potentially develop without a concomitant elevation of the chick's RMR levels.

### *Homeothermic Chicks*

For chicks that reached homeothermy only RMR values were determined. An ANCOVA was used to compare the separate allometric relationships between body mass and RMR for the two lines and for the three feeding levels. It was found that the interaction term line × feeding level × logarithm of the body mass was not statistically significant ($P = 0.39$). After deleting this interaction term from the model, it was found that the interaction terms line × logarithm of the body mass ($P = 0.48$), and feeding level × logarithm of the body mass also were insignificant ($P = 0.13$). After having deleted these terms from the model, it appeared that the line effect was not significant ($P = 0.25$). Consequently, after deleting this term, the logarithm of the body mass ($P < 0.001$) and feeding level were found to contribute significantly to the model ($P < 0.001$).

## Discussion

### *Morphological Development*

The results have shown clearly that the chicks of all groups were well able to cope with food restrictions, even when the chicks received only 40% of the age-specific ad lib. levels. Moreover, we found no difference between the fast-growing and normal-growing lines with respect to their sensitivity to food restrictions: survival rates were high in both lines. Therefore, an interesting conclusion that can be drawn from this observation is that in the Japanese quail the selection for high growth rates has not resulted in a decrease in the level of morphological and physiological plasticity.

We also found that the body masses of the birds at 60 d of age were not directly proportional to their relative feeding level. For example, at this age the birds that received 70% of the ad lib. level exhibited an average body mass of 81% of the ad lib.–fed birds, and the birds that received 40% of the ad lib. level exhibited an average body mass of 50% of the ad lib.–fed birds. Thus, the food-restricted birds have grown at a higher efficiency level. There are numerous plausible explanations for this observation. First, it is well possible that the food-restricted chicks exhibited lower food passage rates through the gastrointestinal tract, which potentially results in a higher assimilation efficiency (Karasov 1996). Second, in food restricted groups, morphological changes may have taken place with respect to the intestinal tissue, potentially resulting in a reduction of the metabolic costs (Karasov 1996). Third, several other metabolic processes may have been reduced in food-restricted chicks, such as the metabolic rate during fasting and/or the level of activity. Fourth, the feeding levels of the food-restricted chicks were determined on the basis of the age-specific ad lib. levels and

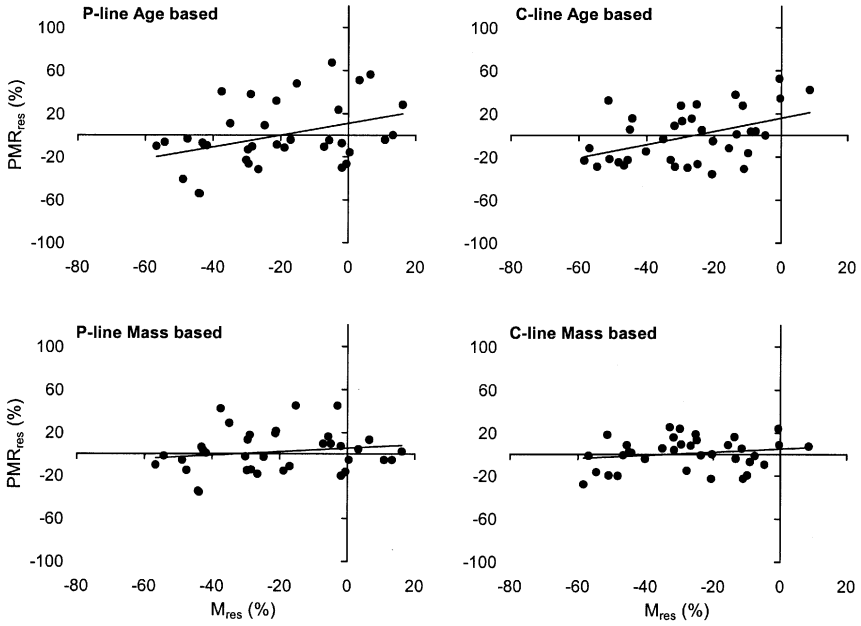


Figure 6. Age- (*upper panels*) and mass-based (*lower panels*) regressions of peak metabolic rate residuals ($PMR_{res}$) and body mass residuals ($M_{res}$) in Japanese quail chicks of the P- and C-line. For P-line chicks, age-based regression refers to Equation (6), but the mass-based regression was not significant. For C-line chicks, age-based regression refers to Equation (7), but the mass-based regression was not significant.

not on the mass-specific ad lib. levels, which has resulted in a relative "overfeeding" of the food-restricted chicks.

Schew and Ricklefs (1998) have shown that Japanese quail chicks (C-line) are immediately able to adjust the growth rates of both wing chord and tarsus in response to a reduction in feeding level to the maintenance level. In our experimental protocols, levels of food restrictions were less severe, resulting in a positive energy balance at all ages. Under these conditions, growth rates of the wings were much less reduced than those of the tarsus and head. For example, in chicks that received 40% of the ad lib. level, the wing length was always at least 75% of that of the ad lib. group. At the end of the experiment, differences with respect to wing length among P-chicks had disappeared. Under natural conditions, preference of wing growth over growth of other structural components may be adaptive, enabling precocial chicks to escape from predators and, thus, to enhance the chicks' chances for survival (Lack 1968). Interestingly, at the end of the experimental period, wing lengths were (almost) identical for all groups, irrespective of the chicks' body masses (see Fig. 1). At this age, this resulted in unusual ratios of wingspan to body mass in the food-restricted birds, and it is questionable whether these birds could fly effectively under windy conditions. However, a similar range in wingspan to body mass ratios has been reported in free-living semiprecocial oystercatcher (*Haematopus ostralegus*) chicks (Kersten and Brenninkmeijer 1995). Oystercatcher chicks typically fledge after having achieved a wing length of 180 mm, irrespective of their body masses (observed range from 190 to 400 g). Although body masses of these fledglings differed tremendously (highest value is 2.1 times the lowest value), variation in the length of the leg and head were much smaller (for both components, the highest value was 1.2 times the lowest value).

During the early phase of the postnatal period, growth of the wing and tarsus follow a linear pattern, whereas growth of some other components (e.g., the pectoral or leg muscle) follows an exponential pattern (Ricklefs 1983; Carrier and Auriemma 1992). Linear growth of the feathers and bone has been explained by the fact that there is only one specific area where cell proliferation takes place (Ricklefs 1983). In contrast, growth of the muscles takes place throughout the entire tissue, resulting in exponential growth rates in young chicks (Ricklefs 1983). In the field, precocial chicks may often experience only short periods of food restriction (e.g., because of spells of inclement weather; Beintema and Visser 1989*b*). Under these conditions, it seems adaptive for a bird to favor the growth of its structural components over the growth of the breast muscles. If food availability improves, growth rates of the muscles can probably be increased rapidly ("catch-up growth"), resulting in a normal chick (Schew and Ricklefs 1998). In some species, like the oystercatcher, it has been demonstrated that catch-up growth occurs after fledging, when chicks are able to feed themselves (Kersten and Brennikmeijer 1995).

Chicks of the altricial European starling lack the ability to

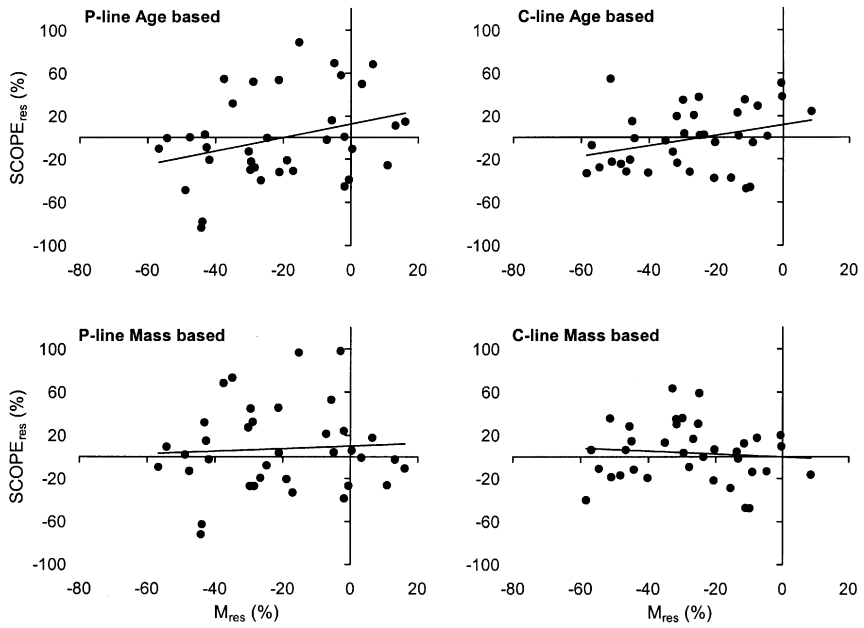


Figure 7. Age- (*upper panels*) and mass-based (*lower panels*) regressions of SCOPE residuals ($SCOPE_{res}$) and body mass residuals ($M_{res}$) in Japanese quail chicks of the P- and C-line. None of the drawn regressions were statistically significant.

stop the growth of wing and tarsus in response to a reduction in feeding level to the maintenance level (Schew and Ricklefs 1998). For example, when being fed on the maintenance level on the first day, growth rates of tarsus and wing were only reduced by about 20%. The authors suggested that the growth of these structural body components continued at the expense of the energy and nutrient stores. It is unclear whether the differences in responses between Japanese quail and European starling are because of differences in growth rates. If growth rates shape these developmental responses to food restrictions, we would expect strong differences between chicks of both Japanese quail lines. As can be deducted from our results on two lines of the Japanese quail with contrasting growth rates, there is no effect of the growth rate on the degree of plasticity of morphological and physiological development. It has to be noted, however, that Japanese quail chicks have been artificially selected for a high growth rate using selection criteria developed by the poultry industry, such as the achievement of body mass at a specific age or the chicks' level of food conversion at early ages (Marks 1980). It is not sure whether these poultry-induced selection factors are in operation at the same level in free-living chicks of other species. Therefore, more research is urgently needed on the plasticity of morphological and physiological development of free-living species to evaluate whether our results based on Japanese quail chicks are a general phenomenon.

### *Long-term Effects of Food Restriction: Achievement of Sexual Maturity and Survival*

It was demonstrated clearly that achievement of female sexual maturity was closely related to the feeding level, but our experimental setup precluded the determination of the onset of egg laying for each individual. Morse and Vohra (1971) manipulated the food quality of growing Japanese quail starting 1 wk after hatching. Chicks that received deficient food for only a short period started producing eggs at a much younger age, but at a slightly higher body mass, than chicks that received deficient food for a longer period. Apparently, at a given light : dark regime, in laboratory-reared chicks body mass plays a larger role for the achievement of sexual maturity than age. Gebhardt-Henrich and Richner (1998) reviewed the literature with respect to the long-term effects of food restriction in free-living animals. In 20 out of 30 studies that reported long-term effects of food restriction, a positive relationship was observed between body mass at fledging and postfledging survival. In four studies, a positive relationship was found between relative mass at fledging and the subsequent resistence of the birds to cold or starvation. The remainder of the studies reported relationships between body mass at fledging and acquisition of territory, clutch size in the first breeding season following fledging, and recruitment rate of the first breeding season following fledging.

*Physiological Development*

When compared at the whole-body level, age-specific resting and peak metabolic rates of the food-restricted groups were much lower than those of the ad lib.–fed groups. This resulted in remarkable differences in the timing of homeothermy. For example, the ad lib.–fed chicks were homeothermic at about 1 wk of age. However, in chicks that received only 40% of the ad lib. level, achievement of homeothermy was delayed until about 3 wk of age. When in the wild, undernourished chicks would need parental warming for much longer than the ad lib.–fed chicks. This would not only result in a reduction of the time available for foraging but would also make food-restricted chicks more vulnerable to disturbance until achievement of thermal independence (Visser 1998). However, a reduction in the level of energy expenditure may also be advantageous for undernourished chicks because, under these conditions, they would need to consume less food to cover their maintenance requirements.

Because growth rates of food restricted chicks were lower than those of chicks fed ad lib., we are able to (partially) disentangle the separate effects of age and body mass on the development of resting and peak metabolic rates. In chicks of the Arctic tern (*Sterna paradisaea*), it was found that the relative levels of peak metabolic rate were strongly reduced if their body masses were below 25% of the average levels (Klaassen and Bech 1992). This would suggest the presence of a body mass window for normal thermoregulatory development. If body masses of (semiprecocial) Arctic tern chicks are 25% below the average levels, thermoregulatory capacity apparently collapses. Interestingly, both lines of the Japanese quail exhibited a much higher degree of plasticity of physiological development. In chicks of both lines, a normal thermogenic heat production capacity was observed even if body masses were 55% below the average line-specific ad lib. levels (Figs. 6, 7). This suggests a much wider window for normal thermoregualtory development in the precocial Japanese quail chicks than in the semiprecocial Arctic tern chicks. Unfortunately, at present, no other data are available to make a comparison with other species, but we assume that the relatively high degree of developmental plasticity observed in the Japanese quail chicks may be related to their precocial mode of development. Self-feeding chicks may need to allocate more of their ingested energy to the development of their thermoregulatory capacity than parentally fed chicks to give them sufficient time to obtain food. But more comparative data are needed to test this idea.

*Relationship between Relative SCOPE and Relative Body Mass*

In both strains, no relationship could be found between the relative level of metabolic scope and the relative body mass of the chick. The chick's SCOPE has often been considered as a measurement for the functional maturity of the its muscles (Ricklefs 1979). On the basis of this interpretation, it can be concluded that the development of functional maturity (relative to the chick's age) is not related to the mass of the chicks (relative to the chick's age).

*Relationship between SCOPE and RMR*

In both lines, we were unable to demonstrate a relationship between $RMR_{res}$ and $SCOPE_{res}$, suggesting that $SCOPE_{res}$ may develop without a concomitant increase of the chick's $RMR_{res}$. It has been argued that chicks exhibiting high SCOPE levels may require larger organs associated with food processing (such as the stomach, gastrointestinal tract, liver, and intestines) to support such high levels of energy expenditure (Klaassen and Drent 1991). Because these tissues exhibit high mass-specific metabolic rates, a capacity to sustain high PMR levels may also lead to an elevation of the chick's RMR. Clearly, our findings did not support this notion. In addition, the capacity for a high metabolic scope may require muscles with a higher degree of functional maturity, which may also result in an elevation of the chick's RMR. Apparently, in Japanese quail chicks, a relatively high SCOPE level can develop without a concomitant change in their RMR level. Clearly, these issues can only be resolved when combing measurements of metabolic rates, muscle development, and enzyme activities (see Ricklefs 1979).

## Acknowledgments

Gerard Overkamp and Polly Boon were most helpful during the start of the experiments. Karen Krijgsveld and Robert Ricklefs made many improvements on an earlier draft of this manuscript. This work was partly supported by grant OPP-9696134 (National Science Foundation) to R. E. Ricklefs.

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